61

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: 3/3

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

	Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2005/009343	30.08.2005	30.08.2004

International Patent Classification (IPC) or both national classification and IPC
C07K16/06, B01D15/26, B01D15/38, B01D15/36

Applicant
LONZA BIOLOGICS PLC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Lechner, O Telephone No. +49 89 2399-8687

Form (PCT/ISA/237) (Cover Sheet) (January 2004)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2005/009343

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material:

      ☐ in written format

      ☐ in computer readable form

   c. time of filing/furnishing:

      ☐ contained in the international application as filed.

      ☐ filed together with the international application in computer readable form.

      ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2005/009343

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2-12
	No:	Claims	1, 13-21
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-21
Industrial applicability (IA)	Yes:	Claims	1-21
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

see form 210

Item III

Item V

1 Reference is made to the following documents:

D1 JOSIC-D ET AL.: "Analytical and preparative methods for purification of antibodies" FOOD TECHNOL BIOTECHNOL, vol. 39, no. 3, 2001, page 215-226, XP002357256

D2 FAHRNER R L ET AL: "INDUSTRIAL PURIFICATION OF PHARMACEUTICAL ANTIBODIES: DEVELOPMENT, OPERATION, AND VALIDATION OF CHROMATOGRAPHY PROCESSES" BIOTECHNOLOGY AND GENETIC ENGINEERING REVIEWS, INTERCEPT LTD., ANDOVER, GB, vol. 18, July 2001 (2001-07), pages 301-327, XP008034714 ISSN: 0264-8725

D3 US-B1-6 399 750 (JOHANSSON INGEMAR) 4 June 2002 (2002-06-04)

D4 BONNERJEA JULIAN: "Purification of therapeutic proteins." METHODS IN MOLECULAR BIOLOGY (CLIFTON, N.J.) 2004, vol. 244, February 2004 (2004-02), pages 455-462, XP009057988 ISSN: 1064-3745

D5 "Antibody purification (Handbook, 18-1037-46, Edition AC)" 2002, AMERSHAM BIOSCIENCE

D6 WO 2004/076485 A (LONZA BIOLOGICS PLC; BONNERJEA, JULIAN; PRENETA, ANNA) 10 September 2004 (2004-09-10)

2 Novelty (Article 33(2), PCT)

2.1 **D1** discloses that protein-A (PtA) bound Ab are most commonly eluted by low pH buffer such as 0.1 M glycine-HCl or 0.1 M citric acid using a step gradient. Neutralization of the eluate preserves biological activity. An additional ion-exchange chromat. step (IEX) can be utilized to remove the non-specific Ig. Ab aggregates bind to PtA with higher affinity than momomeric Ab, thus, linear gradient pH elution can partly resolve Ab aggregate etc. from the monomeric Ab. The most widely recognized concern with PtA purification is Ab denaturation that can manifest as aggregation, fragmentation and loss of biological activity due to harsh elution conditions (c.f. p221, rhc) . **D1** teaches that IEX has been a platform for Ab purification for many years. IEC separates proteins based on differences in the surface charge of the molecules. Because Ab molecules have a ore basic pI than the majority of other serum or contaminating proteins, IEX is useful in purifying Ab regardless of isotype. The general strategy in IEX is to keep the pH below pI Ab so that they will not bind to the anion exchanger, or, alternatively, to raise the pH above the pI where the Ab will bind to the e.g. DEAE-groups. The opposite strategy works for cation

exchangers. Due to the fact that every Ab is unique and can vary in its pI, binding to an IEC resin needs to be explored and determined experimentally on an individual basis (c.f. p 219, lhc, §2-3). High single step purity requires narrow peak cutting that can reduce recovery significantly (c.f. p 220, lhc, §1).

*Although **D1** does not explicitly mention fractionation, it is implicit to chromatography, that the eluate is fractionated in order to be able to select those parts of the eluate with the highest purity etc.*

Thus, in view of the teachings of **D1**, the subject matter of **claims 1, 13-16, 18-21** cannot be considered novel in the sense of **Article 33(2), PCT**.

2.2 **D2** discloses a method comprising the step of Ab purification by PtA affinity chromatography, which however, does not clear aggregates and adds PtA into the pool (c.f. p 306, §2). In a 2nd step cation exchange chromatography clears Ab aggregates, leached PtA and host cell proteins, which eluate in the regeneration phase after the monomeric Ab. Finally anion exchange chromatography is employed. The Ab yields are PtA >95%, KIEX >75%, AIEX >95%, resulting in an overall process yield of 65% (c.f. p 308, I §3). When developing elution conditions, the balance between purity, yield and peak with may result in elution conditions where the aggregate is not baseline resolved from the Ab. In this case, rather than eluting in the regeneration, some aggregate will elute in the tail of the main Ab peak. When this occurs, special attention needs to be paid to the pooling conditions so that an Ab peak low in aggregate can b collected. Often, by ending the pool at a relatively high absorbance, a low-aggregate peak can be collected without greatly affecting yield. The conductivity of the PtA affinity chromatography pool is reported to be low (< 5mS/cm) (c.f. p 315-316). **D2** discloses a method yielding at least 99% amount of monomer in the pools produced by change of conductivity (c.f. p 319, §3). In case of anion exchanger running the column pH just below Ab binding will allow the least dilution so that the column is typically run at a pH that is 0.5-1 units below the Ab pI (c.f. p 322, §4).

*Although **D2** does not explicitly mention fractionation, it is implicit to chromatography, that the eluate is fractionated in order to be able to select those parts of the eluate with the highest purity etc.*

Thus, in view of the teachings of **D2**, the subject matter of **claims 1, 13-17, 21** cannot be considered novel in the sense of **Article 33(2), PCT**.

2.3 None of the prior art documents at hand explicitly disclose the subject matter of **claims 2-12** which, therefore, has to be considered novel in the sense of **Article 33(2), PCT**.

3 Inventive step (Article 33(3), PCT)

The subject matter of **claims 2-12** would not appear to involve an inventive step in the sense of **Art. 33(3), PCT** for the following reasons:

D3 is considered to be the closest prior art and discloses a separation medium having a base matrix and matrix-bound groups which exhibit recombinant PtA containing a Cys. The groups are of formula:where B is a bridge which binds to the base matrix and X includes a heteroatom N or S from rPtA-Cys. X may be a thioether sulphur and/or a secondary amine (-NH-) or a PtA variant with a C-terminal Cys.

The specific embodiments of **claims 2-12** do not add subject matter which would appear to involve an inventive step in the sense of **Art. 33(3), PCT** in view of the closest prior art **D3** and the general knowledge of a skilled person in the field (c.f. e.g. **D1**, **D4** or **D5**).

4 further remarks

4.1 **Claims 1-21** would generally appear to lack essential technical features in the sense of **Art. 6, PCT**, as they only mention the goal of an individual step but not the technical means on how to achieve said goal, i.e. pI of the Ab to be purified, buffers, flow rates, etc. etc.

4.2 Expressions like "preferably", "for example", "such as" or "more particularly" are considered to have no limiting effect on the scope of claims (e.g. **claims 14, 19, 20**); that is to say, the feature following any such expression is to be regarded as entirely optional (c.f. **Guidelines 5.40**).

4.3 No document corresponding to the cited document Good-NE (1986, Biochemistry 5:467-476) could be found in the literature (c.f. p 17, l 1).

item VI

The following document has been found being relevant for novelty upon entry into the

European regional phase:

D6 (WO2004076485) discloses a method of purifying Ab (IgG) comprising the steps of:

1) purifying an Ab (e.g. #5) by PtA affinity chromatography using as elution buffer 0.1M glycine/HCl, pH 4.0 and neutralizing fractions comprising the Ab peak with a suitable buffer. The PtA contamination in the eluate amounts to 1,6 µg/mg Ab after diafiltration

2) loading the purified Ab on an ion-exchange material. AB solution is loaded onto the column and the flow through collected e.g. by means of fractionation of the flow through.

3) fractionating the flow through and harvesting from the flow through of the ion exchanges at least one monomeric Ab fraction having reduced PtA contents (c.f. p 20, p 22-29;table 2; table 8, 9; p 30-33; claims 1-20). Under certain conditions, fractionation of rPtA is observed across the main elution peak as shown in table 5. Careful pooling of fractions is therefore required to ensure good clearance of rPtA